

-continued

ATC ATC CGC AAC
Ile Ile Arg Asn
404

1212

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid (synthetic DNA)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CAACATGTCG TCACTCATAT GTGTTTCCTG TGTGAAATT

39

What is claimed is:

1. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;
creatine+H₂O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5–10.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

2. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;
creatine+H₂O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

3. The creatine amidinohydrolase of claim 2, which is obtained from *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375).

4. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;
creatine+H₂O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

5. The creatine amidinohydrolase of claim 4, which is obtained from *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374).

6. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;
creatine+H₂O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

20 K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

25 7. The creatine amidinohydrolase of claim 6, which is obtained from *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).

8. A method for producing the creatine amidinohydrolase of claim 1, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

30 9. The method of claim 8, wherein said microorganism is selected from the group consisting of *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374), *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375) and *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).

10. A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 1, a sarcosine oxidase and a composition for the detection of hydrogen peroxide.

11. The reagent of claim 10, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore and a buffer.

12. The reagent of claim 11, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase and myeloperoxidase.

13. The reagent of claim 11, in which the chromophore comprises a hydrogen receptor and a coupler.

14. The reagent of claim 13, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

15. The reagent of claim 13, in which the coupler is an aniline derivative or a phenol derivative.

16. A method for determining creatine in a sample, which comprises measuring the absorbance of the pigment produced by the reaction of the reagent of claim 10 with the sample.

17. A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 1, a sarcosine oxidase and a composition for the detection of hydrogen peroxide.

18. The reagent of claim 17, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore and a buffer.

T00230-T0504550

21

19. The reagent of claim 18, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase and myeloperoxidase.

20. The reagent of claim 18, in which the chromophore comprises a hydrogen receptor and a coupler. 5

21. The reagent of claim 20, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

22

22. The reagent of claim 20, in which the coupler is an aniline derivative or a phenol derivative.

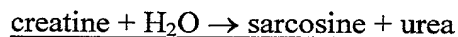
23. A method for determining creatinine in a sample, which comprises measuring the absorbance of the pigment produced by the reaction of the reagent of claim 17 with the sample.]

* * * * *

03404 0330
T 03230 T 460450

24. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

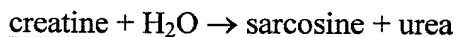


Heat stability: not more than about 50 °C (pH 7.5, 30 min)

K_m values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM.

25. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

K_m values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM.

26. The creatine amidinohydrolase of claim 24, which is stable at pH 5-8 (40 °C, 18 h preservation).

27. The creatine amidinohydrolase of claim 24, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

28. The creatine amidinohydrolase of claim 24, which has a molecular weight of about 43,000 (SDS-PAGE).

29. The creatine amidinohydrolase of claim 25, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

30. The creatine amidinohydrolase of claim 25, which has a molecular weight of about 43,000 (SDS-PAGE).

0340941.033001
T03280" T1601650

31. The creatine amidinohydrolase of claim 26, which has the following physicochemical properties:

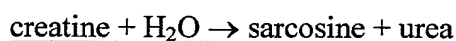
Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

32. The creatine amidinohydrolase of claim 26, which has a molecular weight of about 43,000 (SDS-PAGE).

33. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50 °C

Optimum pH: pH about 8.0-9.0

Molecular weight: about 43,000 (SDS-PAGE).

34. The creatine amidinohydrolase of claim 33, which has the following physicochemical properties:

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Heat stability: not more than about 50 °C (pH 7.5, 30 min).

35. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM.

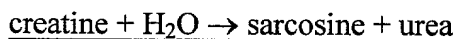
Optimum temperature: about 40-50 °C

Optimum pH: pH about 8.0-9.0

Molecular weight: about 43,000 (SDS-PAGE).

36. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM.

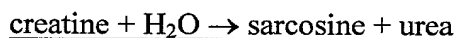
Optimum temperature: about 40-50 °C

Optimum pH: pH about 8.0-9.0

Molecular weight: about 43,000 (SDS-PAGE).

37. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50 °C

Optimum pH: pH about 8.0-9.0

Molecular weight: about 43,000 (SDS-PAGE).

38. A method for producing the creatine amidinohydrolase of claim 24, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

39. A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 24, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

40. A method for determining creatine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 39 with the sample.

41. A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 24, sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

42. A method for determining creatinine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 41 with the sample.

094041 092301
T08280 T460460